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REGENERATION OF THE PROBOSCIS OF MURICID GASTROPODS AFTER AMPUTATION, WITH EMPHASIS ON THE RADULA AND CARTILAGES

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Boring of prey shells by muricid gastropods involves the close interaction of the proboscis, propodium, and the accessory boring organ (ABO) in a predictable cycle which repeats itself continuously throughout the process of boring of each borehole. Although the radula serves only a minor part in shaping the borehole, it appears that both the radula and the ABO are necessary to effect normal penetration (Carriker and Van Zandt, 1972). Whether this is so could be tested by inactivation of one and then the other organ in different individuals and noting the effect on penetration. The first objective of the present study was to examine the effect of removal of the proboscis on boring.

In 1957 an adult *Urosalpinx cinerea* in our laboratory caught its proboscis between the glass and shell of an oyster model (Carriker and Van Zandt, 1972), and after tugging for some time to free itself, tore away the proboscis leaving the distal third wedged in the model. The snail recovered and resumed boring of prey in 20 days. The previous year Demoran and Gunter (1956) experimentally amputated the distal portion of the proboscis of several thaisid boring snails, *Thais haemastoma*, and reported that these gastropods regenerated the proboscis within three weeks provided the organ had been cut off cleanly. From time to time the senior author has dissected large adult *Urosalpinx cinerea* collected in the field in which the anterior portion of the proboscis, though abnormally small, externally appeared morphologically normal. The small size of the proboscis tip indicated that these snails had accidently lost and regenerated the proboscis. These several observations suggest that regeneration of the complex buccal mass in *Urosalpinx cinerea* (Carriker, 1943) and other predatory marine gastropods may occur normally in nature. The second objective of this investigation was thus to determine how commonly and at what rate regeneration occurs in the laboratory after experimental proboscisectomy. A preliminary summary of the results of these studies was published by Carriker (1959, 1961). The radula, being a relatively hard structure (Carriker and Van Zandt, 1972) provides a readily measurable parameter for quantitative determination of regeneration.

During the past several decades regeneration of cartilages has been investigated almost exclusively in vertebrate animals. Recently, however, following a long period of neglect, invertebrate cartilages again came under serious study (Person and Philpott, 1969; Mathews, 1968). As part of a survey of the nature and prop-

erties of cartilages in various invertebrate phyla, Person became interested in their regenerative capacity. In the reports by Demoran and Gunter (1956) and Carriker (1961) neither specific mention nor details were given concerning regeneration of the odontophoral cartilages which were removed by proboscisectomy. The third objective of the investigation was therefore to ascertain whether complete histological regeneration of these structures occurs following proboscisectomy.

Materials and Methods

Healthy well fed individuals of *Urosalpinx cinerea* (Say), *Urosalpinx cinerea follyensis* Baker, and *Eupleura caudata etterae* Baker (Family Muricidae, Class Prosobranchia) were employed in the investigation. Three separate experiments were run. The first was carried out during the summer of 1958 at the University of North Carolina Institute of Fisheries Research, Morehead City, North Carolina, and employed *Urosalpinx cinerea* from the local sounds of North Carolina, and *Eupleura caudata etterae* from Chincoteague Bay, Virginia. The second (1965) and third (1970) experiments were conducted at the Marine Biological Laboratory and employed only *Urosalpinx cinerea follyensis* from Wachapreague, Virginia.

Snails were shipped airmail and maintained in the laboratory in rapidly running seawater. They were fed oysters and acclimatized readily to laboratory conditions. At the Institute of Fisheries Research the temperature of the running seawater during the experiment ranged between 23.5 and 33.0° C, and the salinity between 27 and 32‰. At the Marine Biological Laboratory in 1965 the temperature of the running seawater varied between 18.0 and 22.5° C, the salinity between 31.9 and 32.0‰ and the pH between 8.09 and 8.11. In 1970 the temperature fluctuated between 20.3 and 23.8° C, the salinity between 31.3 and 31.5‰ and the pH between 8.03 and 8.14. During the daytime snails were illuminated by daylight coming through the laboratory windows, and during the early part of the evening by standard overhead artificial laboratory light.

The technique devised for proboscisectomy was developed in the summer of 1957, and was as follows: Into a tray of running seawater we placed a kitchen-type plastic dish, approximately 7.5 by 7.5 by 10 cm, whose sides had been perforated many times to allow ready passage of seawater. A plastic screen, pore size approximately 1 mm, had been cemented into the dish to provide an upright diagonal divider, creating two triangular compartments. In the upstream compartment we placed a freshly shucked live oyster with its flesh against the screen; in the downstream compartment we placed four to six snails. After several hours one or more snails were attracted to the oyster, and after mounting the screen and inserting their proboscides through the screen, they began to feed on the oyster. After the snails had been feeding for a few hours, they could be pulled gently away from the screen without detaching the everted proboscis from the oyster, thereby extending the proboscis further. The considerable length of the proboscis when fully everted, about the same as the height of the snail shell, facilitated the operation. An iris scissors was then carefully inserted between the screen and the snail, and the proboscis was cut quickly and cleanly close to its base, removing with it the buccal mass and more or less of the radular sac depending on the plane of amputation (Fig. 1) and the degree of extension of the sac. The snails were then allowed to recuperate undisturbed in running seawater. Excision of the buccal mass, radula, supporting

odontophoral cartilages, and other structures of the proboscis were verified by examination of the amputated proboscis with a dissecting microscope. For the details of normal morphology of the adult proboscis, see Carriker (1943). No mortality resulted from the proboscisectomies, and all snails recovered rapidly.

The 1958 experiment was designed to determine (a) the number of days after proboscisectomy when boring would resume, and (b) the rate of anatomical regeneration of the proboscis after proboscisectomy. A total of 64 snails was successfully proboscisectomized, 32 snails for (a) and 32 for (b) (see below). Each of (a) and (b) included 16 *Urosalpinx cinerea* (8 males and 8 females, 4 medium and 4 large individuals of each sex) and 16 *Eupleura caudata etterae* (8 males and 8 females, 4 medium and 4 large individuals of each sex). The *Urosalpinx cinerea* ranged in shell height from 16.5 to 25.9 mm, and the *Eupleura caudata etterae* from 19.6 to 34.2 mm.

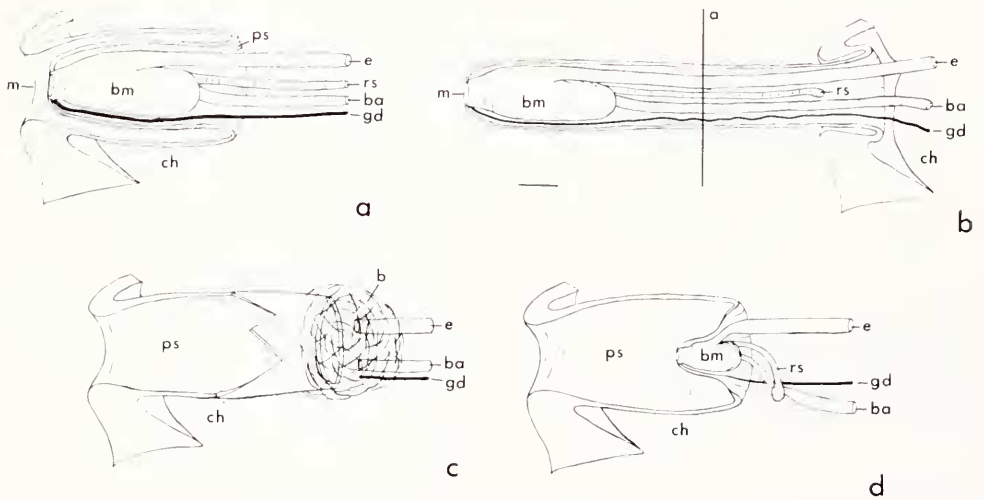

FIGURE 1. Diagrams illustrating the gross anatomy, amputation, and regeneration of the proboscis of *Urosalpinx cinerea,* shell height approximately 25 mm: (a) median plane of the proboscis retracted within the proboscis sac in the cephalic hemocoel; (b) line a, approximate plane at which proboscis amputated; (c) blastema joining the proboscis stump and ends of esophagus, buccal artery, and accessory salivary gland duct; (d) regenerated proboscis tip, buccal mass, and radular sac; a, plane of amputation; b, blastema; ba, buccal artery; bm, buccal mass; ch, cephalic hemocoel; e, esophagus; gd, accessory salivary gland duct; m, mouth; ps, proboscis sac; rs, radular sac; scale bar, 1 mm.

In (a), immediately after proboscisectomy, snails were placed in small translucent perforated plastic dishes in running seawater filtered through coarse sand and shell fragments, each snail isolated with a live oyster 5–7 cm long and cleaned of encrustations. After remaining quietly in a corner of the dish for a day or so, each snail crawled about, and in time was attracted to the oyster and mounted it. Thereafter each day with a needle we gently pushed the midanterior portion of the propodium of the snail back to a point immediately under the ABO. If boring had begun, the initial borehole was clearly evident, the snail was sacrificed, and the

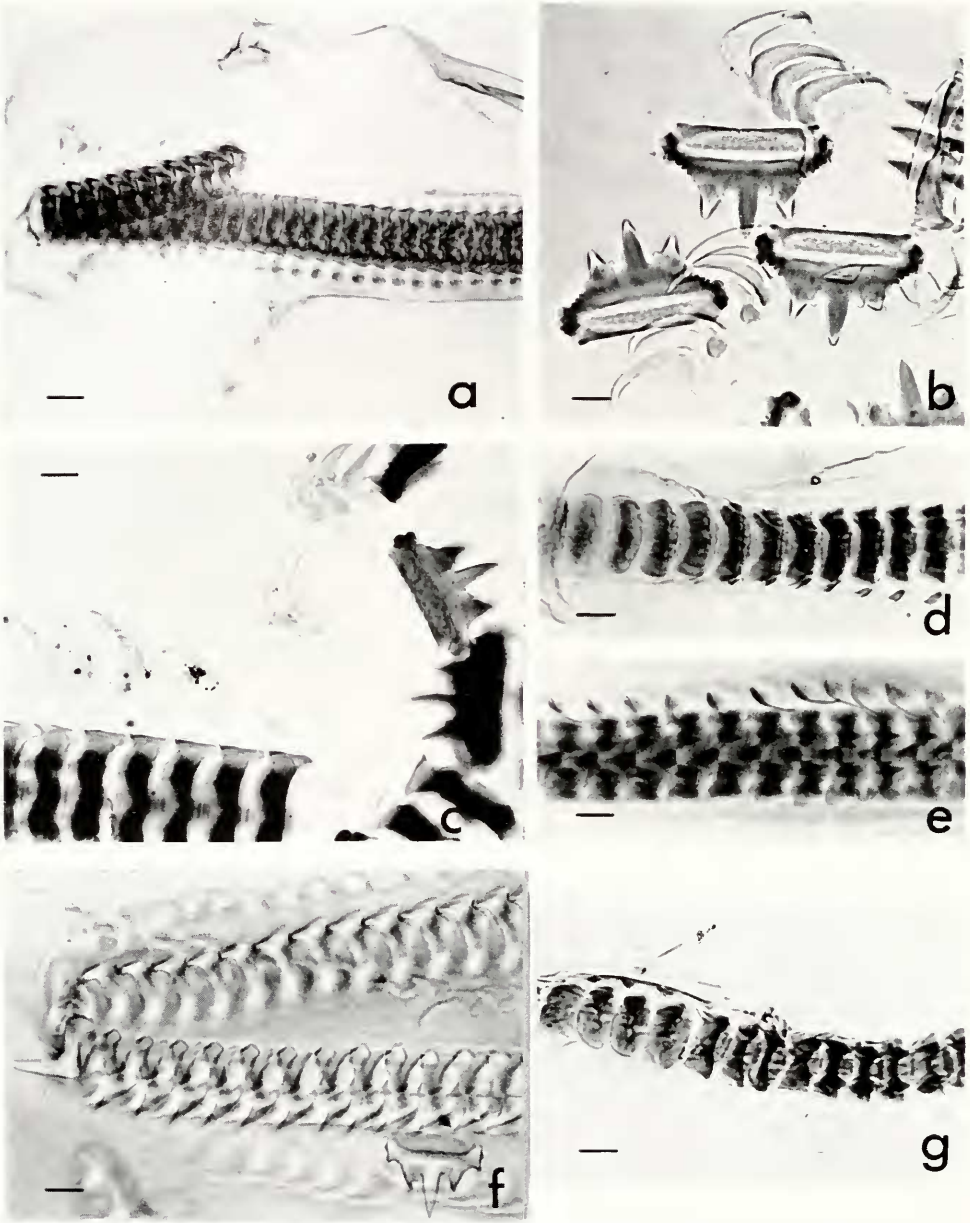

FIGURE 2. a–e: light micrographs of radulae of *Urosalpinx cinerea:* (a) anterior end of normal radula of adult snail on subradular membrane; scale bar, 35 μ; (b) marginal (single cusped) and rachidian (tricusped) teeth of normal radula of adult snail; scale bar, 20 μ; (c) parts of regenerated radula 100 days after snail tore off the anterior third of the proboscis which was wedged in a laboratory device, snail shell height 25 mm; scale bar, 20 μ; (d) anterior portion of regenerating radula 12 days after proboscisectomy; scale bar, 20 μ; (e) posterior portion of same radula as in (d); scale bar, 20 μ. f–g: light micrographs of radulae of *Eupleura*

proboscis was examined for extent of regeneration. If boring had not begun, the snail and oyster were returned to the dish. Oysters were changed weekly with individuals freshly collected in the field. Oysters held in captivity, even in running seawater, tend rapidly to lose their attractiveness after a few days (Carriker and Van Zandt, 1972), possibly because of decreased food.

In (b) proboscisectomized snails were likewise placed in dishes in running filtered seawater, but without oysters. Eight snails (a large and a small male, and a large and a small female of each species) were sacrificed on the 4th, 8th, 12th, and 16th day after amputation, and the regenerating proboscides were examined for degree of anatomical regeneration.

At the time of sacrifice the shell of the recuperating snail was cracked open by a blow with a hammer, and the animal was removed. The regenerating proboscis was then excised from the cephalic hemocoel of the snail, opened under a dissecting microscope, held in position with minute dissecting pins on the exposed surface of a rubber eraser embedded in wax in a dissecting pan, and examined under seawater. Tissues were stained with Little's methylene blue as the dissection progressed. Radulae, if present, were freed of soft tissue in 10% KOH, washed, stained in 1% aqueous chromic acid, dehydrated in alcohols, and mounted flat in Euparol to facilitate examination of the teeth. Because of the tendency of radulae to curl, some of them turned on the side during mounting. Study and measurement of the teeth was done with an ocular micrometer in a compound microscope.

The 1965 experiment was designed to provide regenerating proboscides at intervals of 4, 8, 12, and 20 days after proboscisectomy for histological examination. Thirteen individuals of *Urosalpinx cinerea follyensis,* ranging in shell height from 25 to 43 mm, were utilized, three specimens each for the 4, 8, and 12 day periods, and four for the 20 day period. Regenerating proboscides were everted from the cephalic hemocoel (except in the earliest cases where the proboscis was too small to permit orientation), excised at the base, fixed in cold Bouin's, dehydrated in ethyl alcohols, and embedded in paraffin. Sections were cut 7 μ thick on a standard microtome parallel to the long (anterior-posterior) axis. One half of the sections in each series was stained with Lillie's modification of Masson's trichrome stain, and alternate serial sections were stained with astra blue, Weigert's iron hematoxylin, or Mallory-Heidenhain's stain.

In the 1970 experiment, designed to study the histology and cytology of the regenerating cartilages of the buccal mass, recuperating *Urosalpinx cinerea follyensis* (ranging in shell height approximately from 27 to 38 mm) were sacrificed at time intervals of 3, 7, 11, and 19 days post-proboscisectomy. A minimum of four snails was used in each group. The regenerating proboscides were removed under a dissecting microscope, and half were placed in Bouin's fixative for paraffin embedding, and the remainder were frozen immediately on a quick-freeze stage. Frozen sections 8 μ in thickness were prepared in a Slee freezing microtome for toluidine-blue staining at pH 3. The paraffin embedded sections (5 μ thick) were stained with Wright's nuclear stain, astra blue, or Mallory triple stain.

caudata etterae; (f) anterior end of normal radula on subradular membrane, snail shell height 25 mm; scale bar, 23 μ; (g) regenerating radula 8 days after experimental proboscisectomy, snail shell height 28 mm, anterior portion of the radula to the left, posterior portion to the right; scale bar, 23 μ.

Results

Regenerative changes and rates

In 1958 all 32 individuals of *Urosalpinx cinerea* and *Eupleura caudata etterae* which were allowed to resume boring (experiment a), had regenerated the proboscis, and this organ, though proportionately small, appeared normal under the dissecting microscope. Traces of pink color (probably myoglobin) appeared in the musculature of the buccal mass by the 15th day, and by the 20th day this became more intense. The normal buccal mass in the adult snail is brightly colored. The 32 individuals of both species which were sacrificed at four day intervals after proboscisectomy (experiment b), had all begun regeneration of the proboscis, the degree of development increasing with time past amputation. By the 4th day, at least a filmy cap of loose tissue covered the stump of the proboscis and bound the amputated ends of the esophagus, buccal artery, ducts of the salivary glands, and other tissues to it (Fig. 1c). In two snails (one of each species) the external form of the minute radular sac was already clearly evident. Between the 8th and 12th days after the operation, the four individuals of both species had formed minute normal proboscides and radulae (Fig. 1d), and by the 16th day the radulae had approximately doubled in length.

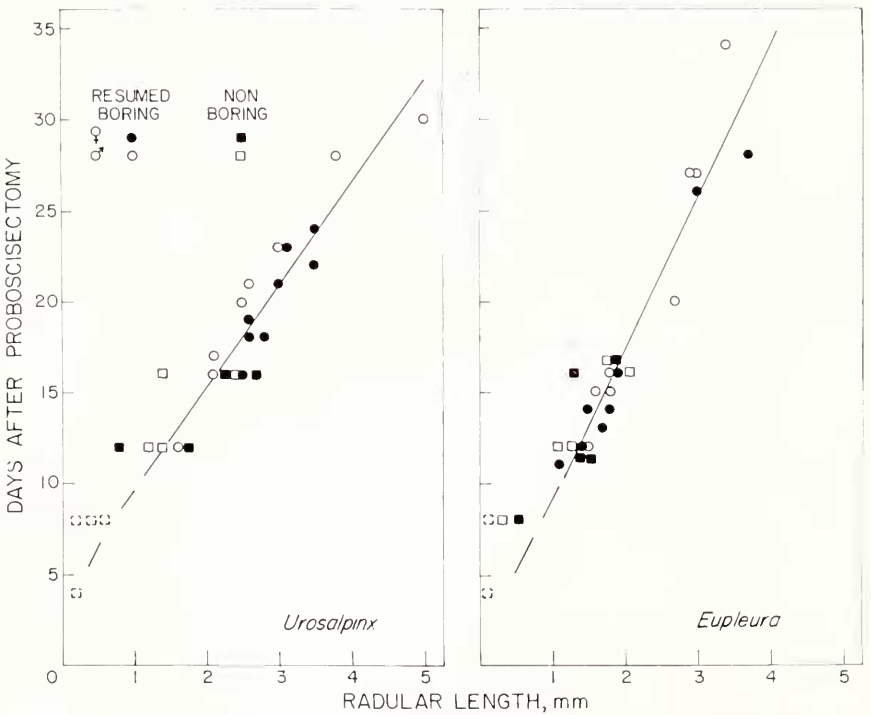

Figure 3. Extent of regeneration of radulae of male and female *Urosalpinx cinerea* and *Eupleura caudata etterae* 4, 8, 12, and 16 days after proboscisectomy ("non boring"), and at resumption of boring after proboscisectomy ("resumed boring").

In two snails, where amputation of the proboscis had taken place at the level of the radular sac, a part of the large original radula, now devoid of its sac but otherwise intact and showing no sign of dissolution, was found in the base of the regenerating proboscis.

In the 8th and 12th day regenerating radulae, the earliest rachidian and marginal teeth were small and slightly misshapen (Fig. 2). In one radula the early mid-rachidian cusp was forked for several initial transverse rows, then in one row changed from the forked to the normal unicuspid condition. Earliest rachidial teeth were separated from each other more than were normal teeth, and even in very small radulae were heavily worn by abrasion, suggesting that the snail starts rasping soon after the teeth are first formed. Teeth increased rapidly in size along the long axis of the radula with time, and successive rows achieved the normal form quickly, the rachidian teeth first, and some time later, the marginal teeth. Earliest regenerating marginal teeth started as short, weak, thread-like structures.

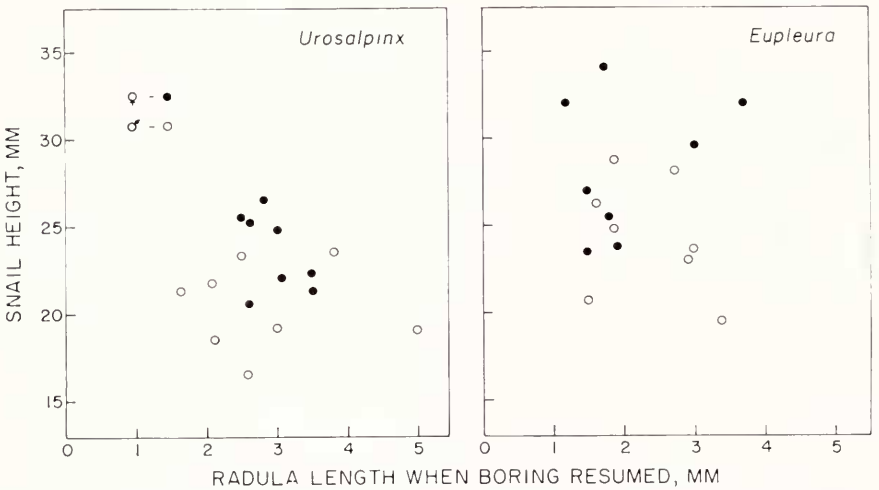

FIGURE 4. Length of radulae and shell height of male and female *Urosalpinx cinerea* and *Eupleura caudata etterae* at resumption of boring.

The approximate rate of regeneration of radulae in *Urosalpinx cinerea* and *Eupleura caudata etterae* is plotted in Figure 3. Initial regeneration was a little faster in *Eupleura caudata etterae* than in *Urosalpinx cinerea*, but the length of the radula increased at a faster rate in some *Urosalpinx cinerea*. There were noticeable differences in the growth rate of radulae among individual snails, but no consistent differences between the growth rate of radulae of males and females.

Although the rate of increase of radulae of both species was surprisingly rapid and relatively uniform, the time of onset of shell penetration of prey varied from 11 to 34 days (Fig. 3). No obvious differences in the onset of boring by males and females was evident. Likewise there was no association between the length of the radula and the height of the snail shell when boring was resumed (Fig. 4).

Measurement with an ocular micrometer of the widest portion of the base of

rachidian teeth at the anterior (oldest) and posterior (newest) ends of (a) normal radulae, (b) regenerating radulae of snails allowed to resume boring after proboscisectomy, and (c) regenerating radulae of snails sacrificed at intervals after amputation, clearly demonstrated the rapid rate of enlargement of teeth with time after proboscisectomy (Fig. 5). However, there was noticeable variation in size between old and new rachidian teeth in some of the normal adult snails of both species: in some, newest teeth were smaller than old ones; in others, they were larger; and in still others, the radula was constant in size throughout its length. Rate of widening of rachidian teeth was maximal in the earliest stages of regeneration of the proboscis (Fig. 5).

Examination of incomplete boreholes excavated by snails which were allowed to bore after regeneration of the amputated proboscis, disclosed that 12 of the holes made by *Urosalpinx cinerea*, and 13 by *Eupleura caudata etterea*, were normal as to size and shape (see Carriker and Yochelson, 1968), and 4 and 3, respectively, were slightly abnormal only in shape. The abnormality, however, appeared to be caused more by the uneveness and irregularity of the growth rings in the oyster shell than by malfunctioning of the radula.

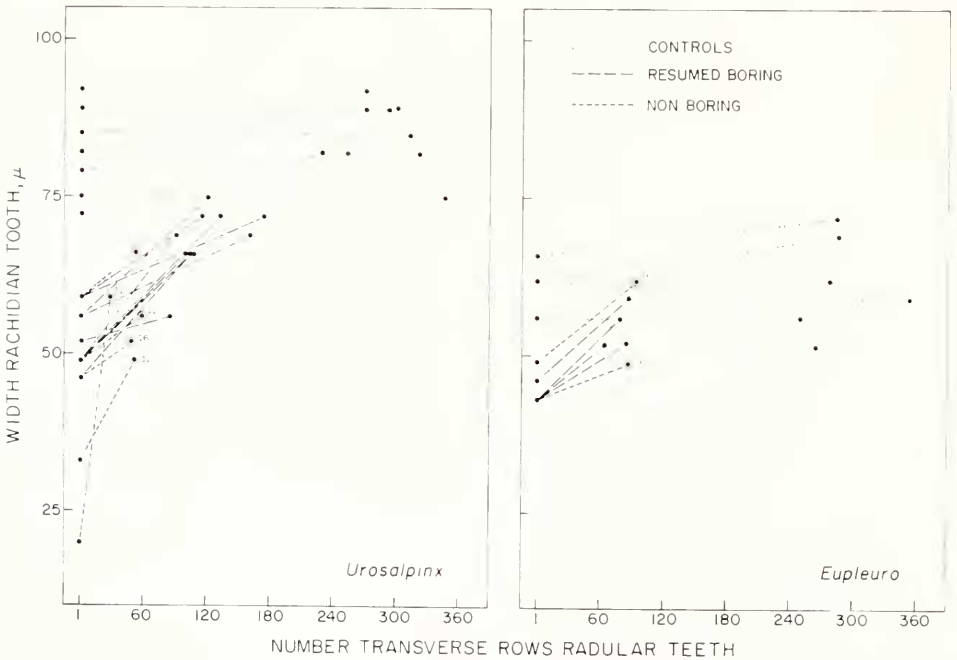

FIGURE 5. Change in width of the base of rachidian teeth of *Urosalpinx cinerea* and *Eupleura caudata etterae* from the anterior (old portion) to the posterior (new portion) of the radulae: in normal snails ("controls"), in snails whose proboscides were amputated and then allowed to regenerate and resume boring ("resumed boring"), and in snails whose proboscides were amputated and then the snails were sacrificed at 4, 8, 12, and 16 days after amputation ("non boring"). Numbers beside points refer to the number of days after proboscisectomy when snails were sacrificed. Teeth were counted from the anterior to the posterior of the radulae.

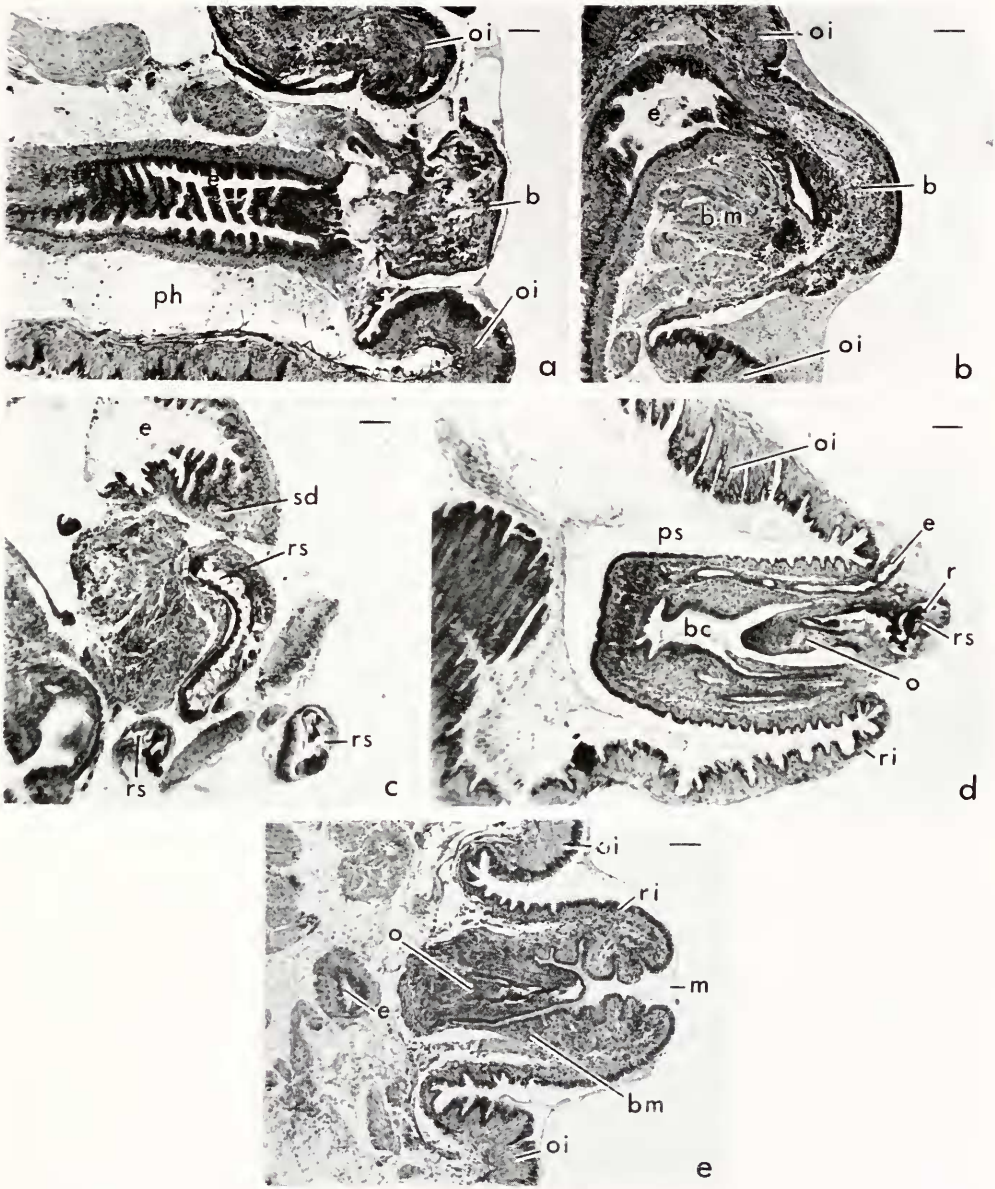

FIGURE 6. Representative stages in the regeneration of the proboscis of *Urosalpinx cinerea follyensis* following proboscisectomy. Snails were sacrificed 4, 8, 12, and 20 days after amputation; light micrographs; Astra blue, Weigert's iron hematoxylin, and Mallory-Heidenhain's stains: (a) initial stage illustrating early blastema, 4 days after amputation; scale bar, 40 μ; (b) early stage, 12 days after amputation; scale bar, 85 μ; (c) intermediate stage showing radula and radular sac, 8 days after amputation; scale bar, 100 μ; (d) new proboscis, withdrawn in proboscis sac, 20 days after amputation; scale bar, 100 μ; (e) new proboscis, everted, 20 days after amputation; scale bar, 100 μ; bc, buccal cavity; b, blastema; c, cap cell; bm, buccal musculature; ct, cartilage, e, esophagus, m, mouth; o, odontophore; od, odontophoral musculature; oi, old proboscis integument; ph, proboscis hemocoel; ps, proboscis sac; ri, regenerating proboscis integument; rs, radular sac; r, radula, sd, salivary gland duct.

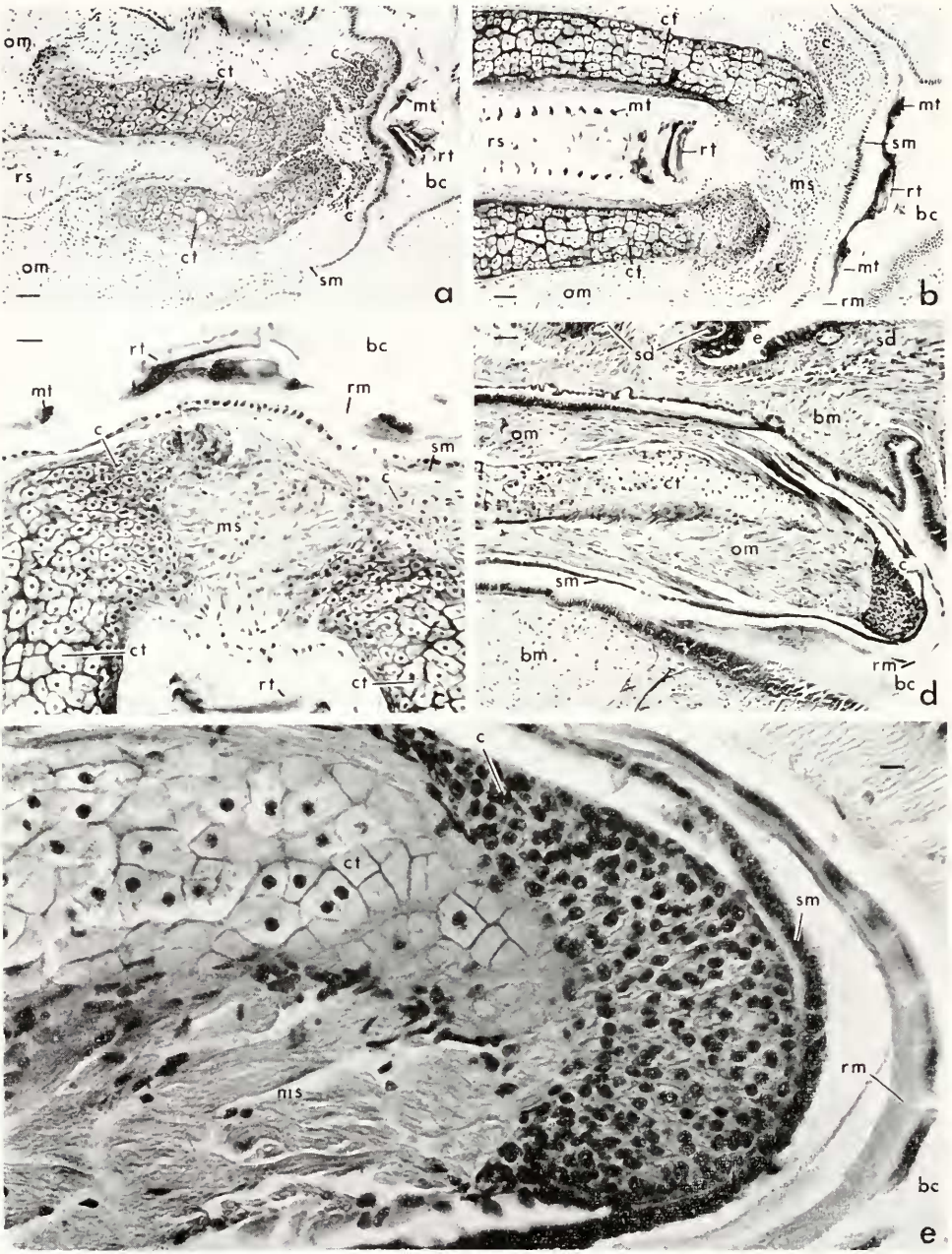

FIGURE 7. Representative stages in normal and regenerating odontophores of *Urosalpinx cinerea follyensis*: (a) frontal section of odontophoral cartilages in nonoperated (control) snail; frozen section, toluidine blue stain; scale bar, 85 μ; (b) frontal section of regenerating odontophoral cartilages in a snail 11 days after proboscisectomy, the section is deeper into the cartilage than in (a), frozen section, toluidine blue stain; scale bar, 85 μ; (c) frontal section of anterior

Histology of regenerating proboscides

Although all 13 proboscisectomized individuals of *Urosalpinx cinerea follyensis* in the 1965 experiment commenced regeneration of the proboscis tip, the rate of regeneration within each time interval varied noticeably, in contrast to the relatively uniform results obtained in the 1958 experiment. Whether this was due to subspecific differences, or to variation in the level of amputation of the proboscis, is not known.

General trends in regeneration of the organs of the proboscis of *Urosalpinx cinerea follyensis* are illustrated in Figure 6. Initially a loose mass of cells, including numerous amebocytes, formed over the cut end of the proboscis and joined this to the cut ends of the esophagus, buccal artery, and other tissues (Fig. 6a). The epithelium and muscular layers of the esophagus then grew forward into the blastema, and the integument of the proboscis extended over the blastema as a thin epidermis one cell thick with mucous cells (Fig. 6b). Simultaneously muscle fibers appeared within the blastema. From this mass arose the integument and musculature of the new proboscis tip, the buccal mass and its musculature, the forward end of the esophagus, salivary and accessory salivary gland ducts, arteries, and nerves, the odontophore, cartilages, radular sac, radula, and cuticular lining of the buccal cavity (Figs. 6c–e, 7d). Odontophoral cartilages, radular sac, and radula were histologically distinguishable by the 8th day after proboscisectomy.

Cytology of regenerating odontophoral cartilages

In the 1970 experiment regenerating radulae and odontophoral cartilages of *Urosalpinx cinerea follyensis* were well advanced by the 7th and 11th days, respectively, after proboscisectomy.

For reference purposes, we will first illustrate the histology of the odontophore of a normal nonoperated *Urosalpinx cinerea follyensis* (Fig. 7a). The cartilages were sectioned in a dorsal peripheral plane of the odontophore, and lie surrounded by the odontophoral musculature (om). The radular sac (rs) emerges posteriorly between the cartilages, and rachidian teeth (rt) are visible on the anterior tip of the odontophore in the buccal cavity (bc). At its anterior end each cartilage possesses a cap of cells (c) which as will be seen, is of complex morphology, and appears closely related to tissues of both the odontophoral cartilages and the surrounding muscle. Nuclei of the cartilage cells stained orthochromatically (blue), whereas the cytoplasm and intercellular matrix of these cells were primarily (but not entirely) strongly metachromatic (pink, purple). Muscle fibers, their nuclei, and

ends of regenerating odontophoral cartilages in a snail 11 days after proboscisectomy. Note details of cap cells and both muscle and cartilage cells issuing from them; frozen section, toluidine blue stain; scale bar, 35 μ; (d) sagittal section through one of the odontophoral cartilages in a snail 20 days after proboscisectomy, illustrating the relationship between the cap, muscle, and cartilage cells; Bouin's fixative, Wright astra blue, and Mallory's stains; scale bar, 85 μ; (e) sagittal section through anterior tip of odontophoral cartilage in a snail 20 days after proboscisectomy, serial section from same specimen as in (d); Bouin's fixative, astra blue, Weigert's iron hematoxylin, and Mallory-Heidenhain's stains; scale bar, 10 μ; bc, buccal cavity, bm, buccal musculature; c, cap cells; ct, cartilage; e, esophagus; ms, muscle cells; mt, marginal teeth; om, odontophoral musculature; r, radula; rm, radular membrane; rs, radular sac; rt, rachidian teeth; sd, salivary gland duct; sm, subradular membrane.

the nuclei of the cap cells were also orthochromatic, while the cytoplasm and intercellular substance of the cap cells likewise exhibited metachromasia.

Regeneration of the cartilages will be described from representative sections of the proboscis from snails 11, 19, and 20 days after proboscisectomy. Figure 7b is a photomicrograph of a frontal section of the odontophore of an 11 day post-proboscisectomized snail. The plane of the section was deeper into the cartilages than was the case in the specimen shown in Figure 7a. It is evident that by the 11th day considerable regeneration of cartilage, radula, and associated tissues had taken place. The distribution of metachromasia and orthochromasia in the tissues appeared similar to that described for the section of Figure 7a, but the metachromasia was more intense. Toward the anterior end of each cartilage in Figure 7b, the cells become smaller in size, and eventually merge with still smaller epitheloid cells which form the cap referred to earlier in the nonoperated snail. At the periphery of the cap, muscle fibers (ms) are in close proximity and appear to interweave with the cap cells (c). This is more clearly illustrated in Figure 7c, a higher magnification of the cap region in a serial section twice removed from that shown in Figure 7b. Both cartilage cells (ct) and muscle cells (ms) blend imperceptibly with the cap cells (Fig. 7c), giving the impression of a blastema-like structure reminiscent of that seen in vertebrate limb regeneration. This impression was strengthened by Figure 7d and 7e which illustrate sagittal sections of regenerated odontophores 20 days after proboscisectomy. At low magnification (Fig. 7d) the cap appears as a tightly packed, rapidly dividing mass of cells from which both cartilage and muscle are forming. At a higher magnification (Fig. 7e) in a serial section twice removed from that shown in Figure 7d, the imperceptible blending of both cartilage and muscle cells with those of the cap is unmistakable, and the resemblance to a vertebrate blastema is reinforced.

Discussion

The ability of muricid gastropods to penetrate the shell of prey allows them, protected by their own shell and for a time by the valves of the prey, to feed on otherwise generally inaccessible organisms often much larger than themselves. After penetration of the shell and while feeding on gaping moribund oysters, however, snails risk loss of the proboscis in two ways: (a) amputation by small crabs and fish when the proboscis is extended into the mantle cavity through the borehole, and (b) pinching and subsequent loss while the proboscis is inserted between the valves. Amputation by both means may occur in nature, though how frequently is not known. Valvular motion of normal live prey inhibits boring between the edges of the valves (Carriker and Van Zandt, 1972), so the danger in (b) is from a prey which after gaping widely for a time suddenly clamps shut, irritated by scavengers feeding on its tissues.

The present studies demonstrated that regeneration of the proboscis takes place in a remarkably short time, and proboscisectomized snails, even the occasional ones in which the amputation is ragged, recover. Rapid functional replacement of the feeding organ insures survival, and an accident which otherwise might have had disastrous consequences, is only a passing inconvenience. The unusual capacity of mollusks to regenerate lost parts has been known for a long time (Hyman, 1967), but the rapid regeneration of so complicated an organ system as the prosobranch

proboscis has not been reported prior to this investigation and the paper by Demoran and Gunter (1956). Isarankura and Runham (1968), by marking the radulae of live pulmonates and prosobranchs (including the muricid, *Thais lapillus*) by various techniques, determined that the rate of replacement (forward movement) of the radula over the odontophore is continuous. The present studies support their findings.

The effect of removal of the proboscis on the capacity of boring snails to penetrate the shell of oysters was demonstrated by individuals of *Urosalpinx* and *Eupleura* which were allowed to resume boring after proboscisectomy. In every case, boring was initiated only after the radula and associated structures had developed normally, anatomically and histologically. Earliest regenerating teeth increased most rapidly in size. Isarankura and Runham (1968) also reported a very rapid rate of replacement of the radula in newly hatched prosobranchs and pulmonates, followed by a steady decrease in replacement rate. Although the rate of increase in length of radulae of *Urosalpinx* and *Eupleura* soon after proboscisectomy was rapid and uniform, the time of onset of boring of prey ranged over a period of 23 days in different individuals. Thus development of a given length of radula did not trigger penetration, and the initiation of boring was stimulated by other factors, possibly attractiveness of prey, or the physiological and behavioral condition of the snails, or both. The noticeable variation in size between old and new rachidian teeth in some adult, nonoperated individuals of both *Urosalpinx* and *Eupleura* was unexpected. Changes, when they occurred, were gradual down the length of the radula, so it is difficult to ascribe them to nutritional causes. Resumption of boring only after the radula and associated structures appeared anatomically normal suggests that the redula is an essential component of the mechanism of shell penetration. Furthermore, the capacity of adult snails with small newly regenerating radulae to excavate boreholes of a shape and size similar to those of normal adult snails is further evidence that the shape and size of the borehole are the products primarily of chemical activity of the accessory boring organ rather than of the radula (Carriker and Van Zandt, 1972).

Regenerating odontophoral cartilages of *Urosalpinx* are strikingly similar in histological appearance to the regenerating limbs of vertebrates, as seen, for example, in the salamander (Butler, 1933; Kiortsis and Trampush, 1965; Thornton, 1968). In both instances the regenerative process is considerably dependent upon a unique cell aggregate, the blastema of vertebrates, and its analogue, which we have called the cap cells, of *Urosalpinx*. Although little is known of the chemistry of the cartilage of these snails, its strong metachromatic staining with toluidine blue at pH 3 indicates the probable presence in the tissue of macromolecular anionic polysaccharides. In *Busycon,* a genus of predatory marine snails, Lash and Whitehouse (1960) reported the presence in the odontophoral cartilage of a nonaminated polyglucose sulfate. Person and Philpott (1963) have also shown that collagen is present in the odontophoral cartilage of *Busycon.* Although at the present time no chemical or ultrastructural data dealing with the odontophoral cartilage of *Urosalpinx* are available, it is likely that some form of anionic polysaccharide and also collagen will be found in its tissue. In view of these findings, and of the relatively rapid regeneration of muricid odontophoral cartilage, we suggest that these and other gastropod families may prove useful for the study of cartilage and skeletal regeneration.

The boring habit and the capacity for rapid regeneration of the proboscis provide unusual advantages in procurement of food, and these perhaps account in large part for the biological success of such muricid species as *Urosalpinx cinerea* and *Eupleura caudata* and their significance as major predators of commercial oysters.

John W. Blake assisted in the investigation in 1958, Barry Martin in 1965, and Robert Lipson in 1970. Photographs resulting from the 1958 and 1965 studies were taken by Peter J. Oldham. The live specimens of *Eupleura caudata etterae* used in 1958 were supplied by Michael Castagna, Thomas Carter, and George Griffith from Chincoteague Bay, Maryland-Virginia; the live specimens of *Urosalpinx cinerea follyensis* employed in 1965 and 1970 were supplied by Michael Castagna from Wachapreague, Virginia.

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Summary

1. All individuals of *Urosalpinx cinerea*, *Urosalpinx cinerea follyensis*, and *Eupleura caudata etterae* from which the proboscis was removed, recovered, and fully regenerated the proboscis. By the 4th day after proboscisectomy a blastema of loose tissue bound the amputated ends of the esophagus, buccal artery, ducts of the salivary glands, and the other tissues to the stump of the proboscis. Between the 8th and 12th days after the operation, snails had formed minute proboscides and radulae. Onset of boring of shell by regenerating snails varied from 11 to 34 days, and took place only after the radula and associated structures were developed and functional. The radula is thus an essential component of the mechanism of shell penetration. Formation of a given length of radula did not trigger penetration; other unexplained factors appear to be responsible.

2. Although earliest regenerating rachidian and marginal teeth were small and misshapen, increase in size and normalization of form was rapid. In time regeneration of proboscides was complete, and they resembled normal proboscides anatomically, histologically, and functionally. Earliest teeth were worn by abrasion, indicating that snails began rasping soon after the teeth and odontophore were formed. Boreholes excavated by snails with small newly regenerating radulae generally corresponded in form and size to those bored by normal snails; this is evidence that the shape and size of the borehole are the products of chemical activity of the accessory boring organ rather than the radula.

3. Histologically the organization of the regenerating odontophoral cartilages and associated musculature and other tissues was similar to that seen in regenerating vertebrate limbs. In both cases the regenerative process is dependent upon a unique cell aggregate (the blastema of vertebrates, and its analogue in muricid

snails), a cap of cells organized at the regenerating tip of the amputated structure.

4. The boring habit and rapid regeneration of the proboscis are distinct assets in procurement of food, and perhaps account in part for the biological success of *Urosalpinx cinerea* and *Eupleura caudata* and their significance as major predators of commercial oysters.

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